mast-cell-deficient mice exhibited reduced early clearance of bacteria in oral infection with *ΔsptP S.* Typhimurium. The defensive role of mast cells identified by this study is in line with these cells' strategic locations in the lamina propria, where mast cells should have a good chance of facing invading bacteria before the bacteria reach mesenteric lymph nodes and the spleen. Use of a protein tyrosine phosphatase with T3SS to block mast cell degranulation was also shown in another Gram-negative bacterium *Yersinia pestis*, which has the homologous phosphatase YopH.

This study has certainly made a major step forward in our understanding of mast cell interactions with *S.* Typhimurium. However, much remains to be learned. A question of immediate interest is how mast cells recognize *S.* Typhimurium (or mediators released from infected cells). The authors note that *ΔsptP S.* Typhimurium enhances Syk phosphorylation induced by stimulation with IgE and anti-IgE, suggesting that without SptP, *Salmonella* might directly activate mast cells. In this regard, a recent study has shown that the absence of TLR11 renders mice more susceptible to *S.* Typhimurium (Mathur et al., 2012). Regardless of direct or indirect mast cell activation, exposure to *ΔsptP S.* Typhimurium appears to involve the phosphorylation of Syk. Another question is related to what the range of SptP targets is and how dephosphorylation of the target proteins leads to biological outcomes such as blockade of degranulation. Although the current study shows a clear reduction in tyrosine phosphorylation of Syk and NSF in SptP-expressing cells compared to control cells, rigorous enzymatic characterization of SptP-mediated dephosphorylation will be required for claiming that these molecules are its direct targets. Interestingly, SptP has a GTPase-activating protein (GAP) domain involved in the recovery of cytoskeleton after *Salmonella* invasion (Fu and Galán, 1999). These observations suggest that the target proteins of phosphatase and GAP activities might be present in proximal subcellular locations or alternatively that the phosphatase activity works early and GAP activity follows in a temporally and spatially different manner. YopH targets remain to be explored. Another interesting point is what effect SptP might have in gut epithelial cells and macrophages, where *S.* Typhimurium can replicate.

In summary, the current study reveals an effective means adopted by *S.* Typhimurium to avoid an innate immune defense mechanism by targeting the central signaling mechanism of tyrosine phosphorylation to shut down mast cells.

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Making Sense of HIV Innate Sensing

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Innate sensing of HIV is important in host control and pathogenesis. In this issue of *Immunity*, Lahaye et al. (2013) demonstrate that HIV capsid-cyclophilin A interactions affect viral cDNA sensing by the DNA sensor cCAS and contribute to differential pathogenesis of HIV-1 and HIV-2.

Innate immune system sensing of HIV infection plays a role in multiple aspects of HIV pathology, including control of infection and killing of $CD4^+$ T cells. This sensing occurs in productively infected cells, nonpermissive resting $CD4^+$ T cells, and other cells like macrophages and dendritic cells (DCs). The HIV-1 restriction factor SAMHD1 is highly expressed in DCs and resting $CD4^+$ T cells and interferes with reverse transcription by reducing dNTP amounts, rendering these cells resistant to infection (Baldauf et al., 2012, Goldstone et al., 2011, Laguette et al., 2011). HIV-2 encodes Vpx, an accessory protein that promotes the degradation of SAMHD-1, allowing both HIV-2 infection of DCs and innate sensing of virus in the DCs that might limit HIV-2 pathogenesis (Figure 1) (Baldauf et al., 2012). Manel et al. (2010) previously

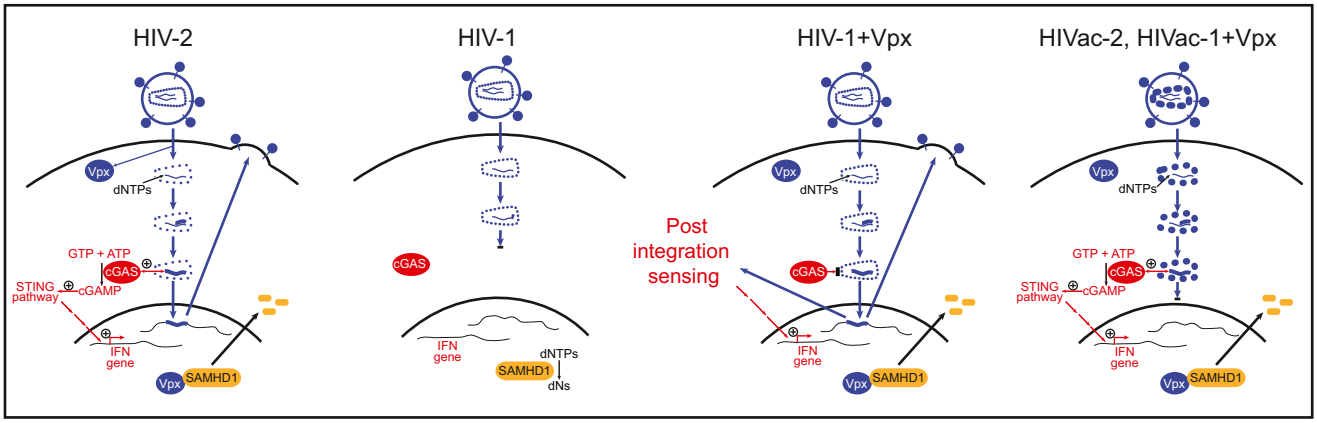

Figure 1. Interactions of HIV-1 and HIV-2 with DC

HIV-2 infection of DCs. DCs can be infected with HIV-2 because the HIV-2 Vpx protein, which is present in the virion, promotes degradation of the host restriction factor SAMHD1. Following reverse transcription, viral cDNA is detected in the cytoplasm by the DNA sensor cGAS. Activated cGAS synthesizes the second messenger cGAMP, which activates the STING pathway, leading to DC activation and a type I IFN response. DCs are not infected by HIV-1. HIV-1 lacks Vpx and therefore cannot counteract the restriction factor SAMHD1. SAMHD1 lowers dNTP amounts and thereby inhibits reverse transcription of viral RNA into cDNA. DC can be infected in experimental situations where Vpx is provided. The infection can be sensed through an incompletely understood postintegration mechanism that depends on the interaction of newly synthesized capsid protein with CpA (Manel et al., 2010). Engineered HIV variants with mutations in the capsid protein that enhance CpA binding (termed HIVac-1 and HIVac-2) are blocked in replication at a preintegration stage, but viral cDNA can be sensed by cGAS.

showed that when DC resistance to HIV-1 infection is circumvented by coinfection with HIV-2 or Vpx delivery via other mechanisms, HIV-1 induces an antiviral type I interferon response and DC maturation. This response is dependent on interaction of HIV-1 capsid with the cellular peptidyl-prolyl isomerase cyclophilin A (CypA), an interaction that also promotes infectivity. The interaction with CypA induces a capsid conformation change that alters viral uncoating. This could in turn affect the ability of intracellular sensors to detect the viral nucleic acid that serves as a pathogen-associated molecular pattern (PAMP).

In this issue of *Immunity*, Lahaye et al. (2013) exploit mutations affecting capsid affinity for CypA to examine the role of capsid in sensing, to identify the viral PAMP for DC sensing, and to dissociate sensing from productive infection given that both are affected by capsid-CypA interactions. HIV-1 and HIV-2 capsid mutations that increased affinity for CypA blocked infection of DCs, but allowed sensing, even for HIV-1, demonstrating that DCs are competent for innate sensing in the absence of productive infection. Capsid-mutated HIV-1 (termed HIVac) was only sensed if Vpx were present. To determine whether viral genetic material is required for sensing, Lahaye et al. used mutations that reduced viral genomic RNA encapsidation. DC sensing was dramatically reduced, demonstrating that normal encapsidation of the genomic RNA is required for sensing. Manel et al. (2010) demonstrated previously that HIV-1 is sensed in the presence of Vpx only after integration. Lahaye et al. (2013) used combinations of HIV mutations and anti-HIV drugs that block reverse transcription, nuclear entry, and integration to demonstrate that HIV-2 is sensed in the cytosol before integration and that production of viral cDNA is required. Mutation of the HIV-2 capsid to enhance CypA binding did not affect sensing. In contrast, mutation of the HIV-1 capsid to resemble the high CpA binding HIV-2 mutant allowed sensing, demonstrating that DCs have the intrinsic capacity to sense HIV-1 DNA and that capsid structure is important in determining where sensing occurs.

The authors then performed genetic targeting of known DNA sensors DDX41, IF116, and cyclic GMP-AMP synthase (cGAS) and demonstrated that the DC sensor in this system is cGAS. Consistent with these results, Gao et al. (2013) recently showed that cGAS senses HIV-1 DNA in the cytosol of a human monocytic cell line. Cytosolic DNA triggers cGAS to produce cyclic GMP-AMP (cGAMP), which binds and activates STING, leading to activation of the transcription factor IRF3 and the production of interferon-β (IFN-β) (Sun et al., 2013).

After entry, the mature viral capsid uncoats, and viral RNA is reverse transcribed into cDNA which then integrates into cellular DNA. The studies cited above suggest that capsid protein structure and interactions with CpA determine whether these steps can take place before the cDNA is sensed by the innate immune system or rendered noninfectious by other processes.

Given that HIV-1 activation of DC innate sensing is abrogated in natural HIV-1 infection by SAMHD1 and by structural features of its capsid, Lahaye et al. suggest that activation of innate sensing might be employed to enhance control of HIV-1 in vaccine design or therapeutically. However, innate immune activation is a double-edged sword. Immune activation is the hallmark of HIV-1 pathogenesis, and there is increasing evidence that innate sensing might play a role in that (Deeks et al., 2013).

Activation of innate immune responses to HIV-1 has different outcomes depending on the cell type. Doitsh et al. (2010) recently used a human lymphoid aggregate culture system to demonstrate that $CD4^+$ T cell death following HIV-1 infection results in large part from abortive infection of nonpermissive resting $CD4^+$ T cells. They demonstrated that the accumulation of reverse-transcribed DNA in these nonpermissive cells elicits a suicidal cellular response, as well as inflammation. Infected cultures produced IFN-β and high amounts of the proinflammatory interleukin-1β (IL-1β). The inflammatory response was completely prevented by

the reverse trancriptase inhibitor efavirenz and by AMD3100, a drug that blocks HIV-1 entry via the HIV-1 coreceptor CXCR4. This suggests that the inflammatory response to abortive HIV-1 infection is triggered by premature termination of viral DNA elongation, which signals caspase-1 and inflammasome activation and the maturation and release of bioactive IL-1β in these $CD4^+$ T cells. Capsase-1 and inflammasome activation are required for IL-1β production in this system. Therefore, caspase-1-dependent cell death, known as pyroptosis, is a plausible mechanism of CD4 depletion.

DNA damage cascades also play a role in death of activated $CD4^+$ T cells that are infected with HIV-1. Cooper et al. (2013) demonstrated that virus-induced $CD4^+$ T cell killing is triggered by integration. Cell death in this system was associated with productive rather than abortive infection. The mechanism of killing following viral integration involved the activation of DNA-dependent protein kinase (DNA-PK), a central integrator of the DNA damage response, which causes phosphorylation of p53 and histone H2AX.

Taken together, these studies highlight the emerging complexity of innate sensing of HIV-1, with multiple pathways in different cell types leading to a variety of outcomes. Lahaye and colleagues speculate that enhancing innate sensing might represent a strategy for enhancing HIV-1 control and producing more effective vaccines. However, manipulation of the innate sensing systems might also result in increased pathogenesis. Therefore, additional studies in this area would be of significant value, particularly in systems that allow interaction between $CD4^+$ T cells and other immune system cells so that the total effects of such manipulations on all cell types and the overall immune response can be determined.

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New Twist on an Ancient Innate Immune Pathway

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Activation of the complement system has long been known to be regulated by a series of steps involving fluid-phase convertases. In this issue of *Immunity*, Liszewski et al. (2013) report the discovery of an intracellular cathepsin-L-dependent C3 activation pathway.

The complement system can be activated by "hard-wired" pattern-recognition receptors (PRRs) that have evolved to recognize pattern-associated molecular patterns (PAMPs). PRRs in the complement system recognize exogenous and endogenous "danger" motifs. Recognition receptors in the complement system (i.e., specific antibody, mannan-binding lectin [MBL], C1q, and natural immunoglobulin M [IgM]) activate three separate complement pathways referred to as the classical, lectin, and alternative. Although each of these pathways is activated by distinct PRRs, they all culminate in activation of the complement factor 3 (C3), the central step in complement activation. The C3 convertase (C3bBb) converts the inactive yet abundant C3 component into the biologically active effector fragments referred to as anaphylatoxins (C3a and C3b). C3a in turn binds its G-protein-coupled receptor, C3aR, on the surface of cells, whereas C3b can either bind its receptor, CD46, or bind to more of the C3 convertase, creating the C5 convertase [C3(H2O)BbP3b], which leads to the generation of C5a and C5b. C5b initiates the terminal enzymatic cascade of the lytic membrane attack complex, which mediates lysis of pathogens and unprotected host cells.

Although liver-generated circulating anaphylatoxins undoubtedly play a role in pathogen control systemically, emerging evidence suggests that anaphylatoxins are also produced by immune cells, including T cells. Once produced, they bind their receptors on the T cell